# PCT

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| **(51) International Patent Classification** [5] : G01N 33/573, C12Q 1/30 | **A1** | **(11) International Publication Number:** **WO 94/22016** |
| | | **(43) International Publication Date:** 29 September 1994 (29.09.94) |

**(21) International Application Number:** PCT/US94/02925

**(22) International Filing Date:** 18 March 1994 (18.03.94)

**(30) Priority Data:**
08/034,935     19 March 1993 (19.03.93)     US

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**(81) Designated States:** AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*
*With amended claims.*

**(54) Title:** SERUM ANTIOXIDANTS AS PREDICTORS OF ADULT RESPIRATORY DISTRESS SYNDROME

**(57) Abstract**

At the initial diagnosis of sepsis (6-24h before the development of ARDS), serum manganese superoxide dismutase (MnSOD) levels and catalase activities are increased in septic patients who subsequently developed ARDS compared to septic patients who did not develop ARDS. Increases in MnSOD and catalase may be used to predict the occurrence of ARDS in septic patients with the same sensitivity, specificity and efficiency as parallel assessments of serum lactate dehydrogenase (LDH) and Factor VIII levels. Evaluation of serum MnSOD and catalase as well as these other accessible markers facilitates identification of subsets of patients and allow prospective treatment of septic patients who are destined to develop ARDS.

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## SERUM ANTIOXIDANTS AS PREDICTORS OF ADULT RESPIRATORY DISTRESS SYNDROME

5                           BACKGROUND
          The present application relates in general
to methods and apparatus for performing assays for
disease states, and in particular to methods and
apparatus for performing assays for adult
10    respiratory distress syndrome (ARDS).
          ARDS is an acute inflammatory process
characterized by lung neutrophil accumulation, lung
edema and progressive hypoxemia [Repine, *Lancet*,
*339*, 466-469 (1992)].  ARDS occurs as a complicating
15    factor in patients with sepsis as well as numerous
other predisposing conditions.  Since many common
and diverse risk factors lead to the development of
ARDS, but ARDS develops only relatively rarely,
pretreating everyone at risk for ARDS is not
20    practical [Fowler et al., *Ann. Intern. Med., 98*,
593-597 (1983)].  Because a better understanding of
ARDS is emerging and various interventions which can
limit inflammation are forthcoming, it has become a
major goal to identify accessible and repeatable
25    markers in at risk patients which predict the
development of ARDS.  This will enable experimental
therapies to be prospectively and effectively
evaluated in smaller, better-defined groups of
patients.

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## SUMMARY OF THE INVENTION

The present invention provides a method
for identifying septic patients for prospective
treatment of adult respiratory distress syndrome
5    including the step of determining a high (greater
than an established baseline) serum level of
manganese superoxide dismutase.

The present invention also provides a
method for identifying septic patients for
10   prospective treatment of adult respiratory distress
syndrome including the step of determining a high
(greater than an established baseline) serum level
of catalase.

According to the present invention,
15   apparatus for identifying septic patients for
prospective treatment of adult respiratory distress
syndrome includes means for determining a high
(greater than an established baseline) serum level
of manganese superoxide dismutase.

20        The present invention also provides
apparatus for identifying septic patients for
prospective treatment of adult respiratory distress
syndrome including means for determining a high
(greater than an established baseline) serum level
25   of catalase.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of MnSOD levels for
healthy control subjects (squares); and of septic
patients who did not develop ARDS (triangles) and
30   septic patients who developed ARDS (circles) at
three times:  at diagnosis of sepsis (1), at
diagnosis of ARDS (2) and after diagnosis of ARDS
(3);

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FIG. 2 is a graph of CAT activity for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at

5      three times:  at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3);

FIG. 3 is a graph of GPX activity for healthy control subjects (squares); and of septic

10     patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times:  at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3);

15     FIG. 4 is a graph of LDH activity for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times:  at diagnosis of sepsis (1), at

20     diagnosis of ARDS (2) and after diagnosis of ARDS (3);

FIG. 5 is a graph of Factor VIII levels for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles)

25     and septic patients who developed ARDS (circles) at three times:  at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3); and

FIG. 6 is a graph of levels of $\alpha_1$Pi-

30     elastase complexes for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times:  at diagnosis of sepsis (1), at diagnosis of ARDS (2)

35     and after diagnosis of ARDS (3).

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In the experiments illustrated in FIGs. 1-6, septic patients were enrolled (0 h) and studied sequentially for the next 48 h. Points were plotted at the diagnosis of sepsis (0 h at 1), at the

5 diagnosis of ARDS (6-24 h after the diagnosis of sepsis at 2) and after the diagnosis of sepsis (6-24 h after the diagnosis of ARDS at 3). Each value is the mean ± SE of 3-20 determinations.

## DETAILED DESCRIPTION OF THE INVENTION

10 In the present investigation, three antioxidant enzymes [manganese superoxide dismutase (MnSOD), catalase and glutathione peroxidase (GPX)] were compared with three other potential markers [Factor VIII [Carvalho et al., *N. Engl. J. Med.*,

15 *307*, 1113-1119 (1982) and Rubin et al., *J. Clin. Invest.*, *86*, 474-480 (1990)] LDH [Ward et al., *J. Clin. Invest.* 76, 517-527 (1985) and Dwenger et al., In: Sturm, ed. *Adult Respiratory Distress Syndrome*, Berlin Heidelberg: Springer-Verlag, 91-127

20 (1991)]] and $\alpha_1$Pi-elastase complexes [Rocker et al., *Lancet*, *1*, 120-123 (1989) and Hilgenfeldt et al., *Eur. J. Clin. Pharmacol*, *38*, 125-131 (1990)] for their ability to predict the development of ARDS in patients with sepsis.

25 Alterations occur in the oxidant-antioxidant balance in ARDS and in other disease states that appear to involve oxygen radicals in their pathogenesis [Leff et al., *Free Radical Biol. Med.*, *13*, 143-149 (1992); Leff et al., *Am. Rev.*

30 *Respir. Dis*, *146*, 985-989 (1992); Buhl et al., *Lancet*, *2*, 1294-1298 (1989); Bernard et al., *Am. Rev. Resp. Dis.*, *139*, A221 (Abstract) (1989); and Pacht et al., *Chest*, *100*, 1397-1403 (1991)]. In addition, patients with established ARDS have

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elevated serum catalase activity [Leff et al., *Am.
Rev. Respir. Dis, 146,* 985-989 (1992)].  Serum
catalase activity increased in a rat model of burn-
induced acute lung injury [Leff et al., *Inflammation*
5   *(In Press)* (1992)].


## EXAMPLE

Patient Consent and Selection.  After
written consent was obtained from the patient or a
family member, each subject was studied using a
10  protocol which was approved by an institutional
human subjects review committee.  All patients
(n=26) who were identified within 8 h of the
diagnosis of sepsis were eligible for enrollment.
Patients with sepsis had a serious bacterial
15  infection and either (a) a rectal or core
temperature exceeding 39°C or (b) a peripheral
leukocyte count of >12,000 cells/$mm^3$ or >20% immature
neutrophils.  Septic patients also had at least one
of the following:  a positive blood culture
20  involving a commonly accepted pathogen, a strongly
suspected or proven source of systemic infection,
gross pus in a closed space, unexplained systemic
arterial hypotension (systolic blood pressure less
than 80 mm Hg), systemic vascular resistance less
25  than 800 dyn x s x $cm^2$ and/or unexplained metabolic
acidosis [Parsons et al., *Am. Rev. Resp. Dis., 140,*
294-301  (1989)].

Patients with ARDS (n=6) met the following
criteria: (1) acute respiratory failure requiring
30  mechanical ventilation, (2) bilateral pulmonary
infiltrates, (3) pulmonary capillary wedge pressure
<18 mm Hg, (4) static pulmonary compliance <50 ml/cm
$H_2O$, and (5) arterial to alveolar partial pressure of
oxygen ratio of <0.25 [Parsons et al., *Am. Rev.*

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*Resp. Dis.*, *140*, 294-301 (1989)]. Serum and plasma
samples were obtained at the diagnosis of sepsis (0
h) and at the diagnosis of ARDS (6-24 h after the
diagnosis of sepsis) and after the diagnosis of ARDS
5    (6-24 h after the diagnosis of ARDS) either through
an indwelling arterial or venous catheter or by
direct venipuncture. Patients were divided into two
groups: septic patients who did not develop ARDS
and septic patients who later developed ARDS.
10   Patients were prospectively and sequentially studied
until death or discharge. All assays were performed
by personnel who were unaware of the diagnoses.
Control subjects (n=15) were healthy individuals.

Source of reagents. Hanks' balanced salt
15   solution (HBSS) was purchased from Gibco
Laboratories (Grand Island, New York). All other
reagents were obtained from Sigma Chemical Company
(St. Louis, Missouri).

Measurement of serum markers. MnSOD
20   [Kawaguchi et al., *Biochem. Biophys. Res. Commun.*
*171*, 1378-1386 (1990)], Factor VIII antigen [Cejka,
*Clin. Chem.*, *28(6)*, 1356-1358 (1982)] and $\alpha_1$Pi-
elastase complexes [Duswald et al., *Surgery*, *98*,
892-899 (1985)] were measured by ELISA. Catalase
25   was assessed by polarographic assessment of $O_2$
evolution [Leff et al., *J. Appl. Physiol.*, *71(5)*,
1903-1906 (1991)]. GPX was measured as the
oxidation of NADPH at 340 nm in glutathione
reductase, glutathione and t-butyl hydroperoxide
30   [Beutler, *A Manual of Biochemical Methods*, Orlando,
Grune & Stratton, Inc., 1-172 (1984)], LDH [Beutler,
*A Manual of Biochemical Methods*, Orlando, Grune &
Stratton, Inc., 1-172 (1984)] and albumin [Corcoran
et al., *Clin. Chem.*, *23*, 765-766 (1977)] were
35   assayed spectrophotometrically. Uric acid was

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measured by HPLC [Terada et al., *J. Appl. Physiol.*,
*65*, 2349-2353 (1988)].

        Statistical analyses. Patient groups were
compared using an analysis of variance with a
5    Student-Newman-Keuls test of multiple comparisons.
An unpaired t test was used to compare the clinical
characteristics of septic patients with or without
ARDS. For calculations of sensitivity, specificity,
positive or negative predictive values and
10   efficiency, 95% confidence intervals were determined
based on the binomial distribution [Cochran, *In:*
*Sampling Techniques,* 2nd ed., New York, John Wiley &
Sons, Inc., 54-59 (1963)]. Significance was
accepted at a p value of <0.05.
15        Clinical Parameters. Septic patients who
subsequently developed ARDS and septic patients who
did not develop ARDS were the same (p>0.05) with
respect to age, gender, hematocrit, hemoglobin,
blood leukocyte count, blood neutrophil count, serum
20   SGOT, bilirubin, albumin, uric acid levels and
APACHE II score [Leff et al., *Ann. Rev. Respir.*
*Dis., 146,* 985-989 (1992); Knaus et al., *Crit. Care*
*Med., 13,* 818-289 (1985)]. The mortality of septic
patients who developed ARDS was 50% (3 of 6)
25   compared to a mortality of 30% (6 of 20) in septic
patients who did not develop ARDS.
        Blood markers patterns. Septic patients
had increased (p<0.05) serum MnSOD levels compared
to control subjects (FIG. 1). However, at the
30   initial diagnosis of sepsis (approximately 6-24 h
before diagnosis of ARDS), septic patients who
eventually developed ARDS had increased (p<0.05)
serum MnSOD levels compared to septic patients who
did not develop ARDS. Serum MnSOD levels remained
35   elevated for the next 48 h in patients who developed

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ARDS while MnSOD levels returned to control levels
during the next 48 h in septic patients who did not
develop ARDS.

5       Similarly, at the diagnosis of sepsis,
serum from septic patients had more (p<0.05)
catalase activity than serum from control subjects.
Again, at the initial diagnosis of sepsis, patients
who later developed ARDS had more (p<0.05) serum
catalase activity than septic patients who did not
10      develop ARDS (FIG. 2). During the next 48 h, serum
catalase activity increased progressively in septic
patients who developed ARDS but did not change in
septic patients who did not develop ARDS.

        In contrast to MnSOD levels and catalase
15      activities, serum GPX activity was essentially the
same (p>0.05) in control subjects and septic
patients regardless of whether ARDS ensued (FIG. 3).

        Serum from septic patients who
subsequently developed ARDS also had increased
20      (p<0.05) LDH activity compared to serum from septic
patients who did not develop ARDS. Serum from
septic patients who did not develop ARDS had the
same (p>0.05) LDH activity as serum from control
subjects (FIG. 4). Serum LDH measurements increased
25      during the 48 h study period in septic patients who
developed ARDS but not in septic patients who did
not develop ARDS.

        Septic patients who did or did not develop
ARDS (FIG. 5) had similarly increased (p<0.05) serum
30      Factor VIII levels compared to control subjects.
Septic patients who did and did not develop ARDS had
similar (p>0.05) Factor VIII levels.

        Finally, plasma $\alpha_1$Pi-elastase complexes
were increased in all septic patients at the initial
35      diagnosis of sepsis but differences between septic

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patients who did or did not develop ARDS were
manifest only at the time of diagnosis of ARDS
(6-24h after the diagnosis of sepsis) (FIG 6).  By
48 h after the initial diagnosis of sepsis, $\alpha_1$Pi-
5    elastase complexes had similarly decreased in septic
patients independent of the development of ARDS.

     <u>Analyses of serum markers.</u>  First, no
correlations were found at any time between any of
the six markers; Second, the positive and negative
10   predictive values and the sensitivity and
specificity of Serum MnSOD levels ($\geq$ 450 ng/ml),
catalase activity ($\geq$ 30 U/ml), LDH activity $\geq$ 250
U/L and Factor VIII levels $\geq$ 445% control were
comparable in predicting the development of ARDS in
15   septic patients (Table 1).  Third, serum MnSOD
levels, catalase and LDH activity exceeded 450
ng/ml, 30 U/ml and 250 U/L, respectively,
approximately 9 h, 12 h and 12 h, on average,
respectively, before the diagnosis of ARDS.  Further
20   results appear in Table 1.

TABLE 1

Comparison of the Sensitivity and Specificity of Blood Markers
as Predictors of ARDS in Septic Patients

| Parameter | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value | Efficiency |
|---|---|---|---|---|---|
| MnSOD ≥ 450 ng/ml | 67% (42-94) | 88% (75-98) | 67% (4-94) | 88% (75-98) | 83% (70-94) |
| Catalase ≥ 30 U/ml | 83% (61-99) | 65% (49-82) | 42% (25-68) | 93% (81-100) | 69% (55-84) |
| GPX ≥ 0.72 U/ml | 50% (27-85) | 47% (31-69) | 25% (12-53) | 73% (53-92) | 48% (34-66) |
| LDH ≥ 250 U/L | 67% (42-94) | 78% (62-92) | 50% (29-81) | 88% (74-98) | 75% (61-89) |
| Factor VIII ≥ 445% Control | 83% (61-99) | 67% (42-94) | 45% (27-73) | 92% (80-100) | 71% (57-85) |
| $\alpha_1$Pi-elastase >940ng/ml | 67% (37-98) | 64% (50-80) | 18% (8-47) | 94% (84-100) | 64% (51-79) |

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In Table 1 each value represents 3-20
determinations at study entry (t=0 h). Values in
parentheses represent 95% confidence intervals.
Also in Table 1; Sensitivity = TP/TP + FN;
Specificity = TN/TN + FP; Positive Predictive Value
= TP/TP + FP; Negative Predictive Value = TN/TN +
FN; and Efficiency = TP + TN/TP + FP + TN + FN.

In Table 1, results are shown for six
sequentially measured factors in the blood of septic
patients who were predisposed to develop ARDS. Nine
to twelve hours before the development of ARDS, two
serum antioxidant enzymes, MnSOD and catalase, were
increased in septic patients who later developed
ARDS compared to septic patients who did not develop
ARDS and that both of these factors predicted the
development of ARDS in septic patients with as good
a sensitivity, specificity and efficiency as
measurements of LDH and Factor VIII. By comparison,
measurements of GPX and $\alpha_1$Pi-elastase complexes were
neither different in septic patients who did or did
not subsequently develop ARDS nor effective in
predicting the development of ARDS in septic
patients.

Assessment of MnSOD and catalase are
useful for defining the pathogenesis of ARDS or
identifying patients with similar pathophysiologies.
Each measurement is accessible, repeatable and
relatively easy to perform. Based on assessment of
these markers, study of prophylactic treatment is
facilitated by reducing the number of at risk
individuals who need to be studied to obtain
patients with ARDS.

Increases in serum MnSOD levels and serum
catalase activity may also have functional
importance. MnSOD and catalase may diminish oxidant

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insults mediated by superoxide anion($O_2^- \cdot$) or hydrogen peroxide ($H_2O_2$) or their products such as hydroxyl radical ($\cdot OH$). This possibility may be especially relevant because accelerated

5   intravascular generation of oxygen radicals from stimulated neutrophils, circulating xanthine oxidase or other sources are implicated in the pathogenesis of sepsis and ARDS [McGuire et al., *J. Clin. Invest.*, *69*, 543-553 (1982); Cochrane et al., *J.*

10  *Clin. Invest.* *71*, 754-758; (1983); Baldwin et al., *Lancet*, *1*, 11-14 (1986) and Grum et al., *J. Crit. Care*, *2*, 22-26 (1987)].

Because the patterns were different for various markers and no two markers correlated with

15  each other, each factor may represent a distinct process and these factors may more correctly reflect various processes occurring in septic patients with ARDS rather than ARDS *per se*. The present work has focused on sepsis-induced ARDS, so different

20  mechanisms may be present in patients who develop ARDS following trauma and other predispositions.

The origins of the factors, although unclear, most likely are multiple. Lung tissue injury is a possible source for increases in LDH,

25  MnSOD, catalase and Factor VIII levels. Endothelial cells are rich in these factors and, if perturbed, may readily increase the levels of these factors in the blood. However, intravascular neutrophil activation may be responsible for increases in $\alpha_1$Pi-

30  elastase complexes because elastase may be present only in neutrophils. Notably, increases in $\alpha_1$Pi-elastase complexes occurred relatively later, at the diagnosis of ARDS, and then decreased by 48 h after the diagnosis of sepsis, which may indicate a

35  decline in neutrophil activity. Red blood cell

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(RBC) hemolysis may be a source for increases in
serum catalase and LDH activity, but not MnSOD or
Factor VIII levels may not, because RBCs do not
contain the latter.  Serum catalase activity is also

5    increased in the serum of rats subjected to skin
burn [Leff et al., *Inflammation* (In Press) (1992)],
and patients with the acquired immunodeficiency
syndrome [Leff et al., *Am. Rev. Respir. Dis, 146*,
985-989 (1992)], but again, in these situations, the

10   source is unclear.  Elevations of IL-1, tumor
necrosis factor (TNF) and endotoxin have been found
in ARDS patients [Parsons et al., *Am. Rev. Resp.
Dis., 140*, 294-301  (1989); Suter et al., *Am. Rev.
Resp. Dis., 145*, 1016-1022 (1992); Siler et al.,

15   *Exp. Lung Res., 15(6)*, 881-894 (1989); Hyers et al.,
*Am. Rev. Respir. Dis., 144*, 268-271 (1991) and Marks
et al., *Am. Rev. Resp. Dis., 141*, 94-97 (1990)] and
may cause increases in antioxidants such as MnSOD
and catalase [White et al., *J. Appl. Physiol., 66*,

20   1003-1007 (1989); Wong et al., *Science, 242*, 941-944
(1988); Brown et al., *Proc. Natl. Acad. Sci. (USA),
86*, 2516-2520 (1989) and Taniguchi, *Adv. Clin.
Chem., 29*, 1-59 (1992)].

Although the present invention is

25   illustrated by the above embodiments, it is expected
that variations and modifications will occur to
those skilled in the art upon consideration of the
present disclosure.  Accordingly, it is intended
that the present invention include all modifications

30   and variations which come within the scope of the
claims.

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WHAT IS CLAIMED IS:

     1.  A method for prospectively identifying septic patients for treatment of adult respiratory distress syndrome comprising the steps of

5    determining a septic patient's serum level of manganese superoxide dismutase, comparing the septic patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum

10   level being predictive of the development of ARDS in septic patients, and identifying a septic patient whose serum level of manganese superoxide dismutase is predictive of the development of adult respiratory distress syndrome.

15       2.  The method of claim 1 further comprising the steps of determining a septic patient's serum level of catalase, comparing the septic patient's serum level of catalase to an established baseline serum level of catalase, said

20   baseline serum level being predictive of the development of ARDS in septic patients, and identifying a septic patient whose serum level of catalase is predictive of the development of adult respiratory distress syndrome.

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3. Apparatus for prospectively
identifying septic patients for treatment of adult
respiratory distress syndrome comprising means for
determining a septic patient's serum level of
5 manganese superoxide dismutase and means for
comparing the septic patient's serum level of
manganese superoxide dismutase to an established
baseline serum level of manganese superoxide
dismutase, said baseline serum level being
10 predictive of the development of ARDS in septic
patients.

4. The apparatus of claim 3 further
comprising means for determining a septic patient's
serum level of catalase and means for comparing the
15 septic patient's serum level of catalase to an
established baseline serum level of catalase, said
baseline serum level being predictive of the
development of ARDS in septic patients.

5. The method of claim 1 wherein the
20 patient's serum level of manganese superoxide
dismutase is determined by ELISA.

6. The method of claim 2 wherein the
patient's serum level of catalase is determined by
assay of serum catalase enzymatic activity.

25          7. The apparatus of claim 3 wherein the
means for determining a patient's serum level of
manganese superoxide dismutase comprises an ELISA
assay.

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8.  The apparatus of claim 4 wherein the means for determining a patient's serum level of catalase comprises an assay for catalase enzymatic activity.

5        9.  A method for predicting the development of adult respiratory distress syndrome in septic patients comprising the steps of determining a patient's serum level of manganese superoxide dismutase, comparing the patient's serum
10      level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum level being predictive of the development of ARDS, and identifying a patient whose serum level of manganese
15      superoxide dismutase is predictive of the development of adult respiratory distress syndrome.

        10.  The method of claim 9 further comprising the steps of determining a patient's serum level of catalase activity, comparing the
20      patient's serum level of catalase activity to an established baseline serum level of catalase activity, said baseline serum level being predictive of the development of ARDS, and identifying a patient whose serum level of catalase activity is
25      predictive of the development of adult respiratory distress syndrome.

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11. An apparatus for predicting the development of adult respiratory distress syndrome in septic patients comprising means for determining a patient's serum level of manganese superoxide
5   dismutase, and means for comparing the patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum level being predictive of the development of ARDS.

10          12. The apparatus of claim 11 further comprising means for determining a patient's serum level of catalase activity and means for comparing the patient's serum level of catalase activity to an established baseline serum level of catalase
15   activity, said baseline serum level being predictive of the development of ARDS.

13. The method of claim 9 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

20          14. The apparatus of claim 11 wherein the means for determining a patient's serum level of manganese superoxide dismutase comprises an ELISA assay.

15. The use of an ELISA to determine the
25   level of manganese superoxide dismutase in a sample for predicting the development of adult respiratory distress syndrome in septic patients.

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16.  A product for predicting the
development of adult respiratory distress syndrome
in septic patients, said product containing an ELISA
for determining the level of manganese superoxide
5    dismutase in a sample of the patient's serum.

17.  A kit for predicting the development
of adult respiratory distress syndrome in a septic
patients using a sample of the patient's serum
comprising:
10          a) a first monoclonal antibody having a
high specific immuno-reactivity against human
manganese superoxide dismutase, said first
monoclonal antibody capable of forming a first
immuno-reactive complex with manganese superoxide
15   dismutase in the serum;
          b) a second monoclonal antibody having a
high specific immuno-reactivity against human
manganese superoxide dismutase, said second
monoclonal antibody being labelled with an enzyme
20   and being capable of forming a second immuno-
reactive complex with the first immuno-reactive
complex; and
          c) a substrate for the enzyme.

AMENDED CLAIMS
[received by the International Bureau on 7 September 1994 (07.09.94);
original claims 11,12 and 14 cancelled;
original claims 1-4,7-10,16 and 17 amended;
new claims 18-20 added;
other claims unchanged (5 pages)]

1. A method for prospectively identifying the development of adult respiratory distress syndrome (ARDS) comprising the steps of determining a septic patient's serum level of manganese superoxide dismutase, comparing said septic patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS in septic patients, and diagnosing ARDS development potential if said septic patient's serum level of manganese superoxide dismutase is greater than or equal to said established baseline serum level of manganese superoxide dismutase.

2. The method of claim 1 further comprising the steps of determining said septic patient's serum level of catalase, comparing said septic patient's serum level of catalase to an established baseline serum level of catalase, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS in septic patients, and diagnosing ARDS development potential if said septic patient's serum level of catalase is greater than or equal to said established baseline serum level of catalase.

3. An immunologic device for prospectively identifying the development of adult respiratory distress syndrome (ARDS) comprising: a means for immunologically determining a septic
5  patient's serum level of manganese superoxide dismutase; and a means for comparing the septic patient's serum level of manganese superoxide dismutase to a baseline serum level of manganese superoxide dismutase, wherein a serum level of
10  managanese superoxide dismatase greater than or equal to said baseline serum level is predictive of the development of ARDS in septic patients.

4. The device of claim 3 further comprising a means for determining a septic
15  patient's serum level of catalase; and a means for comparing the septic patient's serum level of catalase to a baseline serum level of catalase, wherein a serum level of catalase greater than or equal to said baseline serum level is predictive of
20  the development of ARDS in septic patients.

5. The method of claim 1 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

6. The method of claim 2 wherein the
25  patient's serum level of catalase is determined by assay of serum catalase enzymatic activity.

7. The device of claim 3 wherein the means for determining a patient's serum level of manganese superoxide dismutase comprises an ELISA
30  assay.

8.  The device of claim 4 wherein the means for determining a patient's serum level of catalase comprises an assay for catalase enzymatic activity.

5  9.  A method for predicting the development of adult respiratory distress syndrome (ARDS) in a septic patient comprising the steps of providing serum of said patient, determining said patient's serum level of manganese superoxide

10  dismutase, comparing said patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, a serum level greater than or equal to said baseline serum level being predictive of the

15  development of ARDS, and predicting the development of ARDS if said patient's serum level of manganese superoxide dismutase is greater than or equal to said established baseline serum level of manganese superoxide dimutase.

20  10.  The method of claim 9 further comprising the steps of determining said patient's serum level of catalase activity, comparing said patient's serum level of catalase activity to an established baseline serum level of catalase

25  activity, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS, and predicting the development of ARDS if said patient's serum level of catalase activity is greater than or equal to said

30  established baseline serum level of catalase activity.

13.   The method of claim 9 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

15.   The use of an ELISA to determine the level of manganese superoxide dismutase in a sample for predicting the development of adult respiratory distress syndrome in septic patients.

16.   A product for predicting the development of adult respiratory distress syndrome in septic patients, said product including an ELISA for determining the level of manganese superoxide dismutase in a sample of the patient's serum, and a means for determining the level of catalase in a sample of the patient's serum.

17. A kit for predicting the development of adult respiratory distress syndrome in a septic patients using a sample of the patient's serum comprising:

5  a) a first monoclonal antibody having a high specific immuno-reactivity against human manganese superoxide dismutase, said first monoclonal antibody capable of forming a first immuno-reactive complex with manganese superoxide

10  dismutase in the serum of a septic patient;

b) a second monoclonal antibody having a high specific immuno-reactivity against at least one of said first monoclonal antibody, said first immuno-reactive complex, and human manganese

15  superoxide dismutase, said second monoclonal antibody being labelled with an enzyme and being capable of forming a second immuno-reactive complex; and

c) a substrate for the enzyme that is

20  capable of producing a detectable signal in proportion to the amount of manganese superoxide dismutase in the second immuno-reactive complex.

18. The method of claim 9 wherein the patient's serum level of manganese superoxide

25  dismutase is determined by ELISA.

19. The method of claim 9 wherein said established baseline serum level of manganese superoxide dismutase is 450 ng/ml.

20. The method of claim 10 wherein the

30  established baseline serum level of catalase activity is 30 U/ml.
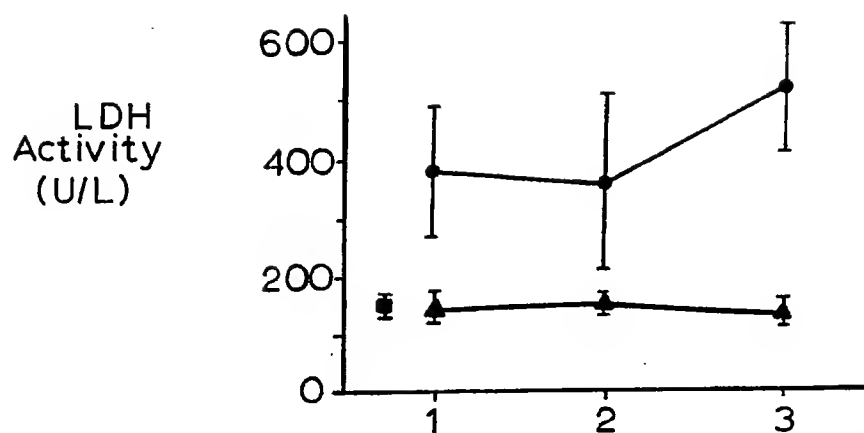
**FIG.1**



**FIG. 2**


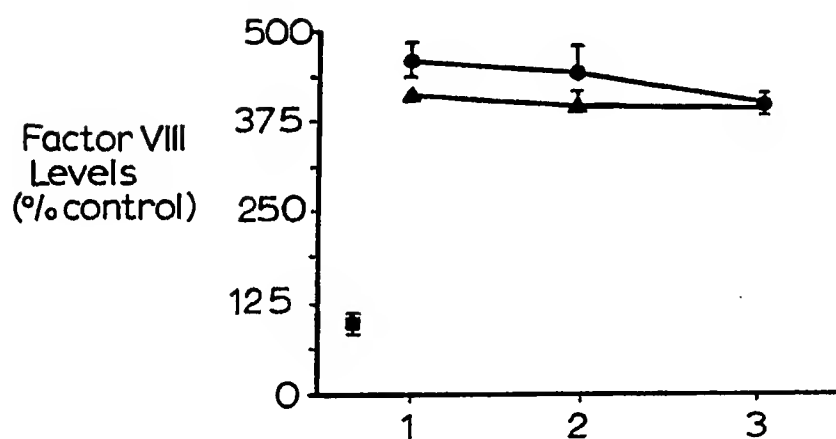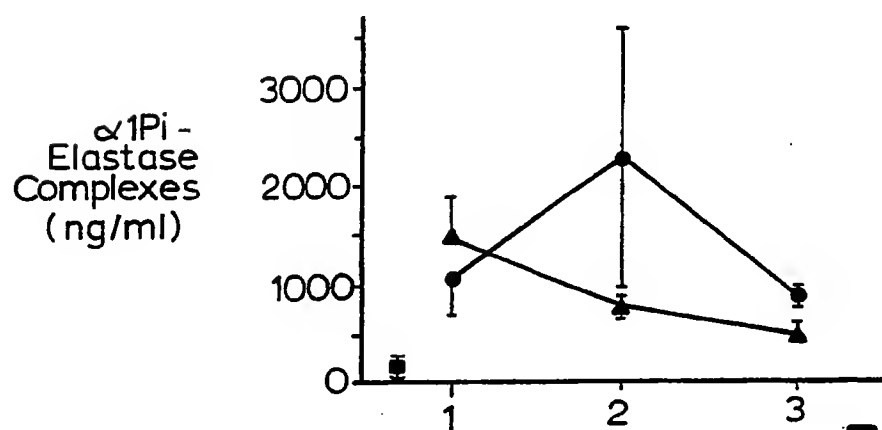
**FIG.3**
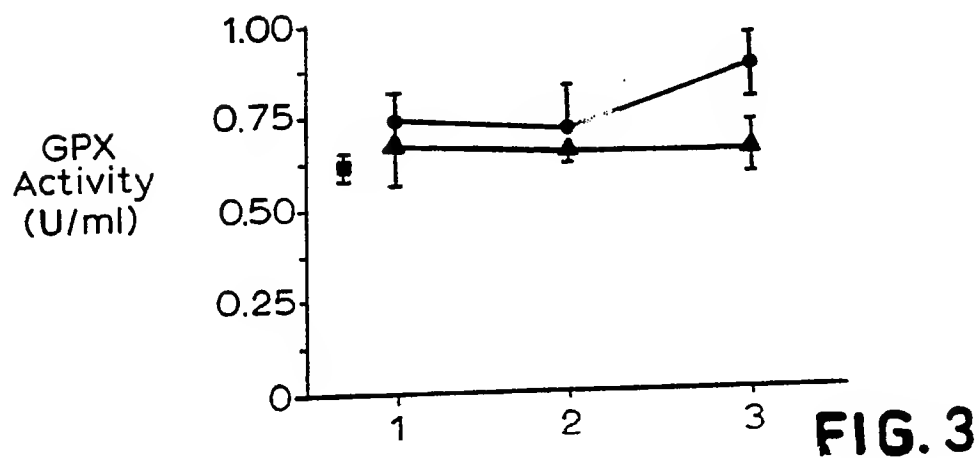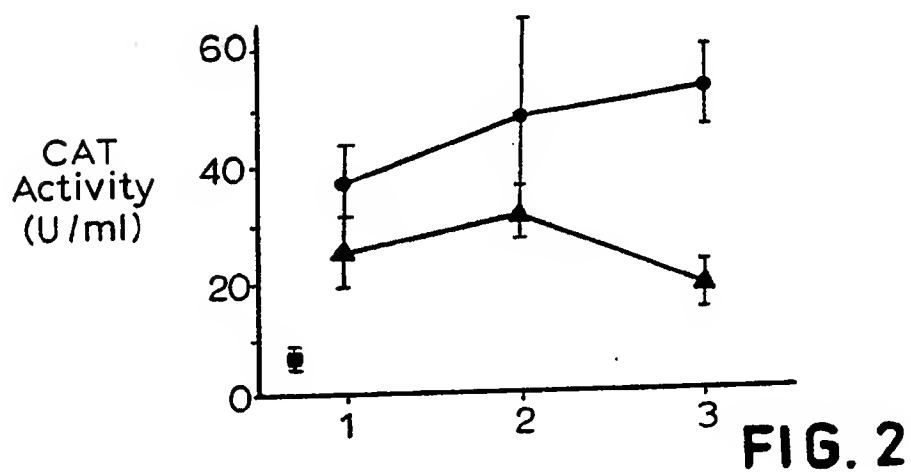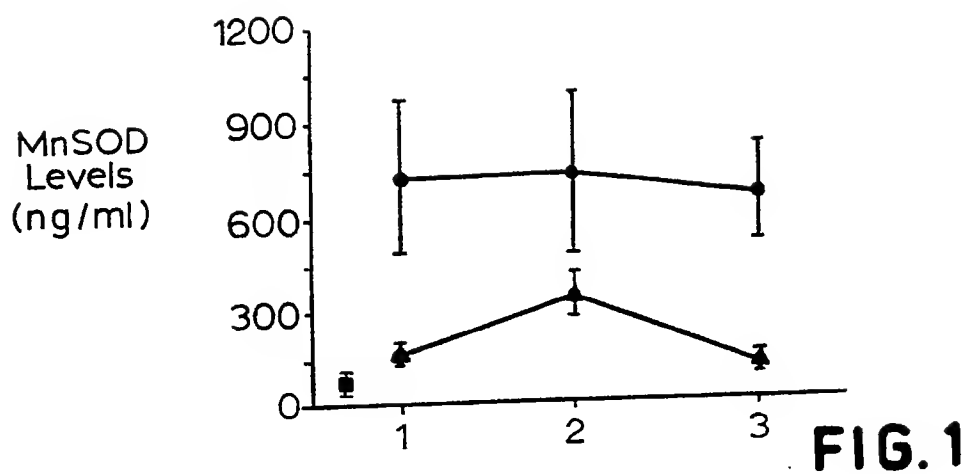
FIG.4



FIG. 5



FIG. 6

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 5    G01N33/573    C12Q1/30

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 5    G01N    C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | CLINICAL RESEARCH<br>vol. 40, no. 1 , 1992<br>page 67A<br>J.A.LEFF ET AL. 'Serum catalase activity is increased in septic patients and predictive of adult respiratory distress syndrome (ARDS) development.'<br>see abstract<br>--- | 2,4,6,8,<br>10,12 |
| X | AMERICAN REVIEW OF RESPIRATORY DISEASE<br>vol. 143, no. 4(2) , April 1991<br>page A805<br>J.A.LEFF ET AL. 'Increased hydrogen peroxide scavenging and catalase activity in serum from septic patients who subsequently develop the adult respiratory distress syndrome (ARDS).'<br>see abstract<br>--- | 2,4,6,8,<br>10,12 |

-/--

[X] Further documents are listed in the continuation of box C.      [ ] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 June 1994 | 11. 07. 94 |

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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax ( + 31-70) 340-3016 | Hitchen, C |

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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X,P | THE LANCET<br>vol. 341, no. 8848 , 27 March 1993<br>pages 777 - 780<br>J.A.LEFF ET AL. 'Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis.'<br>see the whole document<br>--- | 1-17 |
| A | SURGERY GYNECOLOGY AND OBSTETRICS<br>vol. 167, no. 2 , August 1988<br>pages 92 - 98<br>R.J.BLOOM ET AL. 'Endotoxin and pulmonary cell injury'<br>see abstract<br>----- | 1,2 |